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The Clinical Spectrum of Mutations in L1, a Neuronal Cell Adhesion Molecule

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Mutations in the gene encoding the neuronal cell adhesion molecule L1 are responsible for several syndromes with clinical overlap, including X-linked hydrocephalus (XLH, HSAS), MASA (mental retardation, aphasia, shuffling gait, adducted thumbs) syndrome, complicated X-linked spastic paraplegia (SP 1), X-linked mental retardation–clasped thumb (MR-CT) syndrome, and some forms of X-linked agenesis of the corpus callosum (ACC). We review 34 L1 mutations in patients with these phenotypes.

KEY WORDS: L1, hydrocephalus, MASA, mental retardation, neural cell adhesion

L1 AND ASSOCIATED DISEASES

X-linked hydrocephalus (HSAS: hydrocephalus due to stenosis of the aqueduct of Sylvius) described by Bickers and Adams in 1949 and MASA (mental retardation, aphasia, shuffling gait, adducted thumbs) syndrome described by Bianchine and Lewis in 1974 are 2 X-linked neuronal disorders with an overlapping clinical spectrum. Because linkage analysis showed that both disease loci are localized in Xq28 [Winter et al., 1989; Willems et al., 1990], we suggested L1 as a possible candidate gene for both disorders [Willems et al., 1992]. The L1 (L1CAM) gene is located in Xq28 and encodes a neuronal cell adhesion molecule known to play an important role in the development of the nervous system. L1 mutation studies have confirmed this hypothesis because several L1 mutations were found in families with HSAS and MASA syndrome [Rosenthal et al., 1992; Jouet et al., 1993, 1994, 1995; Van Camp et al., 1993; Coucke et al., 1994; Forrest et al., 1994; Fransen et al., 1994, 1995; Vits et al., 1994; Jouet and Kenwrick, 1995]. In addition, L1 mutations were found in families with complicated spastic paraplegia (SP1) [Jouet et al., 1994] and complicated corpus callosum agenesis/dysgenesis (ACC/DCC) [Vits et al., 1994], each family presenting with a MASA-like phenotype. Apart from these molecular studies, clinical studies have indicated the large overlap in the phenotype of the 4 conditions and the presence of several of these phenotypes in a single family [Schrander-Stumpel et al., 1990, 1995; Fryns et al., 1991; Willems et al., 1992]. This proved that HSAS, MASA, complicated SP1, and complicated ACC/DCC are not separate conditions but rather represent overlapping clinical spectra due to mutations in a single gene, L1 (Fig. 1). Therefore, a nosologic separation between those disorders no longer makes sense. Because the most typical and frequent symptoms caused by L1 mutations include corpus callosum agenesis, mental retardation, adducted thumbs, spastic paraplegia, and hydrocephalus, we have referred to this clinical spectrum as the CRASH syndrome [Fransen et al., 1995] (Table I).

L1, also referred to as L1CAM (L1 cell adhesion molecule) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. It consists of various domains, including 6 immunoglobulin and 5 fibronectin type III domains in the extracellular part, 1 transmembrane segment, and a cytoplasmic domain (Fig. 2). Expression is restricted mainly to the axons of outgrowing nerve cells in the central (CNS) and peripheral (PNS) nervous systems and on Schwann cells in the PNS, suggesting a key role in the axon outgrow and pathfinding during embryonic development (Table II). Interactions with the cellular environment occur via homophilic interactions with itself and via heterophilic interactions with axonin-1 (TAG-1), F3 (F11), and phosphocan [Lemmon et al., 1989]. At least a part of the signal transduction appears to occur through activation with fibroblast growth factor receptors (FGFR) [Williams et al., 1994]. L1 most likely interacts with FGFRs through a motif of 5 amino acids between immunoglobulin domains 3 and 4. This domain is present in all 4 FGFR genes and is called the CAM-homology domain (CHD).

The L1 gene spans 16 Kb and contains 28 exons, encoding 1,256 amino acids (Table II). The availability of the complete genomic sequence with the intron–exon boundaries (A. Rosenthal, personal communication) has facilitated exon-by-exon mutation analysis.

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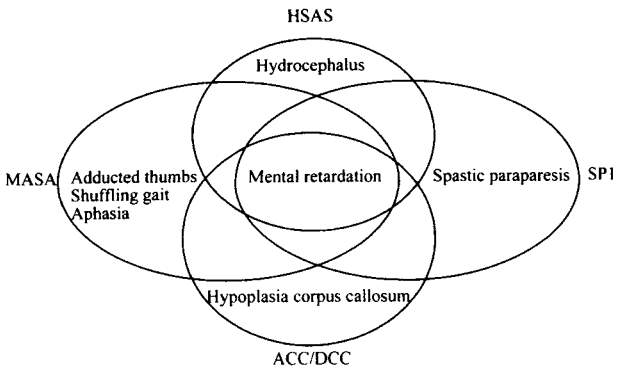

Fig. 1. Schematic representation of the overlapping clinical spectra of HSAS, MASA syndrome, complicated SP1, and complicated ACC/DCC.

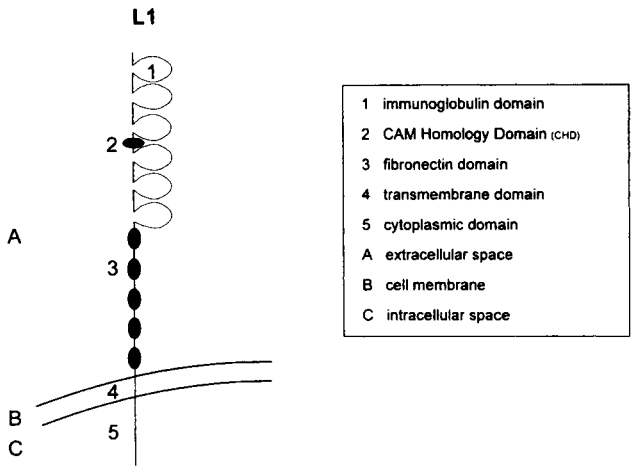

Fig. 2. Model of L1 with its different functional domains.

L1 MUTATIONS

In this paper we describe 7 new mutations and review 27 previously reported mutations [Rosenthal et al., 1992; Van Camp et al., 1993; Jouet et al., 1993, 1994, 1995; Coucke et al., 1994; Forrest et al., 1994; Fransen et al., 1994, 1995; Vits et al., 1994; Jouet and Kenwrick, 1995]. All mutations are described in Table IV and depicted in Figure 3. In many families with HSAS and MASA and in 1 SP1 and 1 ACC/DCC family, an L1 mutation has been identified. These mutations are dispersed over the whole L1 gene: in each structural L1 domain, except for fibronectin III domain 1 and CHD, 1 or more mutations have been found.

The spectrum of mutations shows a wide variety, with gross rearrangements such as large deletions or duplications, small (1–5 bp) deletions or insertions, missense and nonsense mutations, splice site mutations, and a unique and novel type of mutation that destroys a splicing branch point signal [Rosenthal et al., 1992] (Table III). The mutations can be classified into 2 groups. The first group (class I) consists of mutations that disrupt the overall structure of L1 by a nonsense mutation, a splice site mutation, a frameshift mutation caused by a small deletion or insertion, or a larger rearrangement. Approximately half of the currently known L1 mutations belong to this group. The other mutations (class II) are missense mutations. These mutations leave the overall structure of L1 intact in that only a single amino acid is replaced for another (Fig. 3). Class I mutations give rise to a truncated L1 protein if normal transcription, translation, and protein stability are maintained. If the protein is truncated in the extracellular domain, the transmembrane domain is lost, and no L1 can be expressed on the membrane, leading to loss of all functions of L1. If the protein is truncated in the intracellular domain, L1 might still be present on the membrane, and most likely only the intracellular function of L1 is impaired. L1 with class II missense mutations most likely will be present on the cell membrane. However, one of the different functions of L1 will be impaired by the amino acid substitution. If the missense mutation is present in one of the extracellular domains, the binding capacity of L1 with one of its ligands may be disrupted. In vitro constructs with these muta-

TABLE I. Different Designations of Conditions Due to Mutations in L1

HSAS	Hydrocephalus, stenosis of aqueduct of Sylvius
XLH	X-linked hydrocephalus
MASA	Mental retardation, aphasia, shuffling gait, adducted thumbs
MR-CT	Mental retardation–clasped thumbs
ACC-DCC	Agenesis corpus callosum–dysgenesis corpus callosum
SP1	Complicated spastic paraparesis, type 1
CRASH	Corpus callosum agenesis, retardation, adducted thumbs, spastic paraparesis, hydrocephalus

TABLE II. Structure and Function of L1

Structure	Function
Gene: 28 exons (±16 kb)	Migration of neurons
mRNA: 5.5 kb	Outgrowth of neurites
ORF: 1,256 amino acids	Fasciculation of neurites
MW: 142 kDa	Ca^{2+} influx
Domains	Interaction: neuron to neuron, neuron to glial cell
1 Signal sequence (35 AA)	Heterophilic interaction with axonin-1 and NCAM
6 Immunoglobulin domains (579 AA)	Homophilic interaction with itself
5 Fibronectin III domains (499 AA)	Interaction with FGFR
1 Transmembrane domain (30 AA)	
1 Cytoplasmic domain (113 AA)	
Multiple glycosylation sites (Asn-X-Ser/Thr)	
Multiple phosphorylation sites (Thr and Ser)	
1 RGD sequence (Arg-Gly-Asp)	

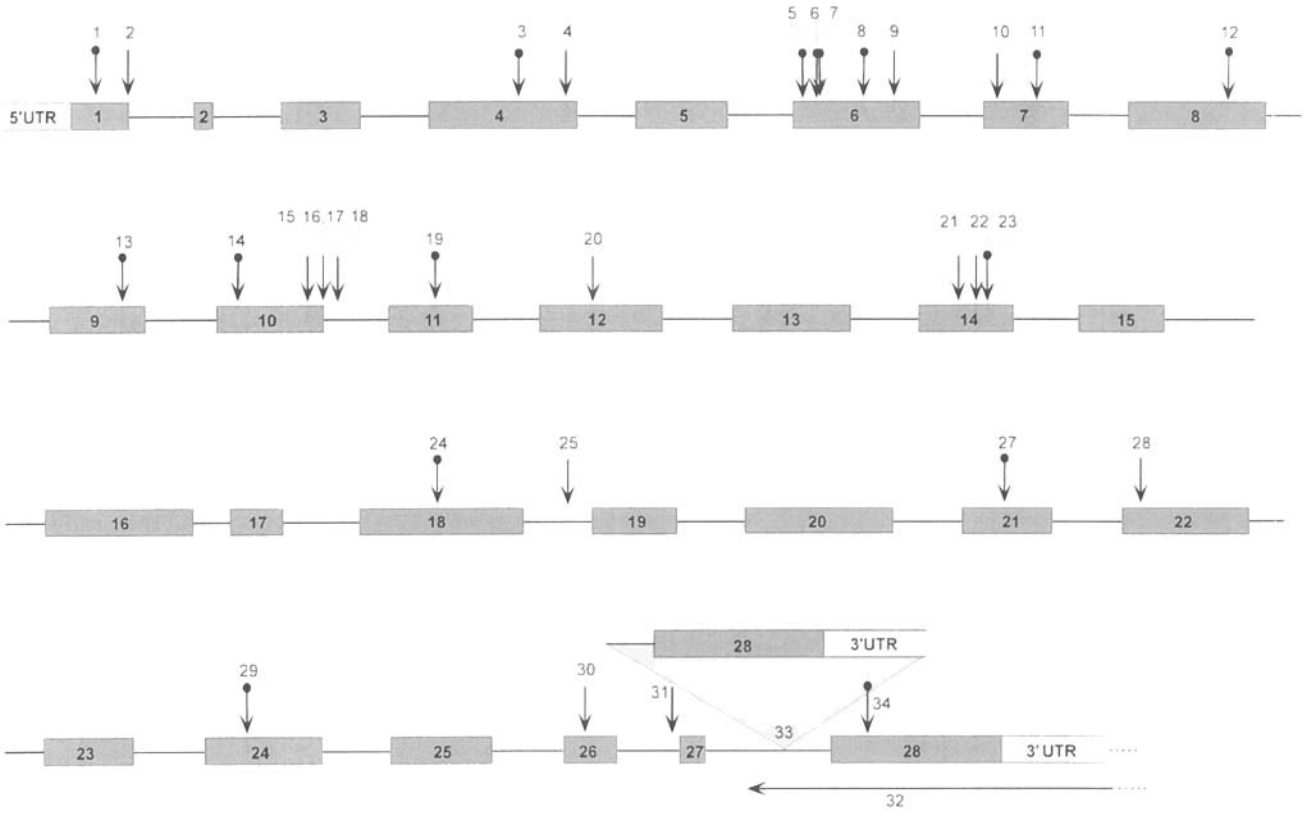

Fig. 3. Genomic structure of the L1 gene. Exons are drawn as boxes, introns as lines. The lengths of all introns and exons 2 (15 bp) and 27 (12 bp) are not drawn to scale. The mutations, numbered according to Table IV, are indicated by vertical arrows. Arrows with a dot represent missense mutations; arrows without a dot represent mutations disrupting the overall L1 structure (stop codon mutations, splice site mutations, frameshift mutations, or large rearrangements).

tions may be used for in vitro studies to elucidate the interactions between L1 and its ligands and the functional L1 domains involved in this binding.

Only 2 mutations in more than 60 families studied in our laboratory can be detected by Southern blot analysis. Therefore, mutation analysis in nearly all families is based on more sensitive diagnostic methods such as exon-by-exon scanning by SSCP or sequencing. However, this is a laborious and time-consuming task because 28 exons have to be investigated.

GENOTYPE–PHENOTYPE CORRELATION

Of the 26 mutations found in families with a severe phenotype (HSAS), 16 belong to class I (mutations disrupting the overall L1 structure) and 10 to class II (missense mutations). In the 8 families with a milder phenotype (MASA, SP1, ACC) 2 have class I mutations and 6 have class II mutations. Although this suggests that mutations disrupting the overall L1 structure more often result in a more severe clinical phenotype including hydrocephalus, this genotype–phenotype correlation is weak. Analysis of the 34 mutations compiled in this paper (Table IV) indicates that different mutations in the same structural domain may lead to different clinical phenotypes. Furthermore, in some cases, the same mutation can lead to different phenotypes within the same family [Schrander-Stumpel et al., 1990, 1995; Fryns et al., 1991; Willems et al., 1992]. In conclusion, it is impossible to predict the severity of symptoms by identification of the L1 mutation.

TABLE III. Different Types of L1 Mutations in CRASH Syndrome

Large deletion	1
Large duplication	1
Small deletion	5
Small insertion	1
Missense mutation	16
Nonsense mutation	2
Splice site	6
Branch point signal	1
Unknown mutation	1
Total number of mutations	34

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TABLE IV. Overview of L1 Mutations*

No.	Exon/intron	Domain	Nucleotide	Consequence	Type	Ethnic origin	Disease	Reference
1	E 1	Signal. pept.	G26→C	W9S	Missense		HSAS	Jouet et al., 1995
2	I 1	Signal. pept.	76 g+1→t	?	Splice site		HSAS	Jouet and Kenwrick, 1995
3	E 4	Ig 1	G361→A	G121S	Missense		HSAS	Jouet et al., 1995
4	I 4	Ig 1	400 g+5 →a	FS 108	Splice site	France	HSAS	Coucke et al., 1994
5	E 6	Ig 2	T536→G	I179S	Missense	Belgium	MASA	Ruiz et al., 1995
6	E 6	Ig 2	C550→T	R184W	Missense	Belgium	HSAS	This study
7	E 6	Ig 2	G551→A	R184Q	Missense	UK	HSAS	Jouet et al., 1994
8	E 6	Ig 2	C630→G	H210Q	Missense	USA	MASA	Vits et al., 1994; Jouet et al., 1994
9	E 6	Ig 2	664 del G	FS 215	Deletion 1 bp	Germany	HSAS	This study
10	E 7	Ig 3	711 ins AGAC	FS 239	Insertion 4 bp	Canada	HSAS	This study
11	E 7	Ig 3	G791→A	C264Y	Missense		HSAS	Jouet et al., 1993
12	E 8	Ig 3	G925→A	E309K	Missense		MASA	Jouet et al., 1995
13	E 9	Ig 4	G1108→A	G370R	Missense	USA	MASA	Ruiz et al., 1995
14	E 10	Ig 4	T1172→C	L391P	Missense	France	HSAS	This study
15	E 10	Ig 4	1248 del T	FS 416	Deletion 1 bp	Hungary	HSAS	This study
16	I 10	Ig 4	1267 g+1→a	skipping exon 10	Splice site		HSAS	Forrest et al., 1994
17	I 10	Ig 4	1267 g+1→a	?	Splice site	Germany	HSAS	This study
18	I 10	Ig 4	1267 a+4→t	Skipping exon 10	Splice site		HSAS	Jouet et al., 1995
19	E 11	Ig 5	G1354→A	G452R	Missense		HSAS	Jouet et al., 1994
20	E 12	Ig 5	C1453→T	R485Stop	Nonsense		HSAS	Jouet et al., 1994
21	E 14	Ig 6	C1756→T	Q586Stop	Nonsense		HSAS	Jouet et al., 1995
22	E 14	Ig 6	1780 del A	FS 594	Deletion 1 bp	U.S.A.	HSAS	This study
23	E 14	Ig 6	G1792→A	D598N	Missense	USA	MASA	Vits et al., 1994
24	E 18	Fn 2	G2302→T	V768F	Missense		HSAS	Jouet et al., 1995
25	I 18	Fn 2	2432 a-19→c	(1) 811 ins 23 AA (2) Skipping exon 19	Branch point		HSAS	Rosenthal et al., 1992
26	E 21	Fn 4	?	Skipping exon 21	?		HSAS	Forrest et al., 1994
27	E 21	Fn 4	C2822→T	P941L	Missense		HSAS	Jouet et al., 1995
28	E 22	Fn 4	2884 del G	FS 962	Deletion 1 bp		HSAS	Jouet et al., 1994
29	E 24	Fn 5	A3209→G	Y1070C	Missense		HSAS	Jouet et al., 1995
30	E 26	Cytopl.	3489 del TG	FS 1164	Deletion 2 bp		HSAS	Jouet et al., 1994
31	I 26	Cytopl.	3531 g-12→a	?	Splice site		MASA	Jouet et al., 1995
32	I 27→28	Cytopl.	3543 del→?	del 1181→end	Deletion 2 kb	Mexico	MASA	Vits et al., 1994
33	I 27→E 28	Cytopl.	3543 dpl 125	FS 1223	duplication 1.3 kb	The Netherlands	HSAS	Van Camp et al., 1993
34	E 28	Cytopl.	C3581→T	S1194L	Missense	The Netherlands	MASA	Fransen et al., 1994

* An updated table of L1 mutations is available electronically through the world wide web at the L1 Mutation Homepage (http://alt-www.uia.ac.be/u/dnalab/l1.html). At this site, a table with every published and many unpublished L1 mutations is maintained, and useful information such as L1 sequences for various organisms, L1 exon structure, L1 domain structure, addresses, and references is listed.

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